# THE STIMULATION OF REPARATIVE REGENERATION OF THE LIVER BY SUBCUTANEOUS INJECTION OF GLYCOGEN

B. P. Solopaev, G. A. Solov'eva, and B. Luzika

Institute of Experimental Pathology and Therapy (Director, Doctor of Medical Sciences B. A. Lapin), of the AMN SSSR, Sukhumi
(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 53, No. 4, pp. 104-108, April, 1962
Original article submitted April 21, 1961

Several workers [3, 4, 5] have found that during regeneration of the liver glycogen content is greatly diminished. We also have observed a marked fall in the glycogen content in the liver of rabbits and monkeys after partial resection of the organ during the period of most intensive increase in weight of the regenerating liver and of increase in the mitotic activity of the liver cells. In control animals subjected to laparotomy without resection of the liver, in order to assess the possible effect of general anesthesia and trauma on the liver, no significant decrease was observed in the glycogen content of this organ [1].

We accordingly decided to investigate the effect of the supplementary injection of glycogen in the period when its concentration falls (the first week after partial resection of the liver) on the course of regeneration of the liver.

## EXPERIMENTAL METHOD

Experiments were conducted on 80 adult male albino rats weighing 200-300 g. The animals were divided into four groups with 20 in each. Partial resection of the liver was performed on all the animals by the method of Higgins and Anderson. The large left and anterior right lobes of the liver, amounting to about $\frac{2}{3}$ of the organ, were removed. The operation was performed under light general anesthesia (ether).

The rats of the first group received daily subcutaneous injections of 4 ml of 6.7% glycogen solution, made up in physiological saline, during the week after operation. The rats of the second group received daily subcutaneous injections of 4 ml of 5% glucose solution during the week after operation. The rats of the third group received daily subcutaneous injections of 4 ml of physiological saline during the week after operation. The fourth group of rats was a control group, and these animals received no additional treatment.

The animals were sacrificed 3, 7, 10, and 14 days after operation, 5 animals from each group at each of these times. A record was made of the weight of the rats before operation, the weight of the resected part of the liver, the weight of the rats at the time of sacrifice, and the weight of the liver at the time of sacrifice, and the weights of the resected part of the liver and the regenerated liver were calculated as percentages of the body weight. The results were treated statistically.

Material for histological section was fixed in Bouin's fluid and 10% formalin solution. The pieces of tissue were embedded in paraffin wax. Sections were stained with hematoxylin-eosin, by Van Gieson's method, and with Sudan III.

The mitotic activity of the liver cells was studied by counting the number of mitoses in 100 fields of vision (aperture of eye-piece 32 $mm^2$, eye-piece 7, objective 90).

## EXPERIMENTAL RESULTS

The results given in Table 1 and in Fig. 1 show that during the first 3 days after operation the weight of the residual part of the liver rose sharply in the rats of all four groups. The relative weight of the liver in the rats receiving glycogen injections was much greater at this time than that in the rats of the remaining groups. It actually reached normal values.

Seven days after operation a further increase in the relative weight of the liver was observed in the rats receiving glycogen and glucose injections, whereas the relative weight of the liver of the rats of the control group and the rats of the group receiving physiological saline remained the same as 3 days after the operation. The relative weight of the liver in the rats receiving glycogen was much greater at this time than in the rats of the remaining groups, including those receiving glucose.

TABLE 1. Changes in the Weight of the Liver in Rats During Regeneration of the Organ

| Time after partial resection of the liver (in days) | Weight of liver (in mg) after injection of | | | |
|---|---|---|---|---|
| | glycogen | glucose | physiological saline | control |
| 3 | 7 306 | 5 685 | 6 450 | 6 060 |
| 7 | 8 930 | 6 062 | 7 364 | 6 766 |
| 10 | 8 033 | 6 168 | 7 280 | 6 598 |
| 14 | 10 990 | 7 105 | 7 420 | 7 412 |

Ten days after the operation the relative weight of the liver of the rats receiving injections of physiological saline, and of the rats of the control group increased slighly by comparison with the relative weight of the liver observed 7 days after operation, but nevertheless was less than in the rats receiving glycogen.

Fourteen days after the operation an increase was observed in the relative weight of the liver in the rats receiving glucose, reaching figures slightly exceeding the normal relative weight of the liver. The relative weight of the liver in the other groups of rats at this time was roughly the same, and was close to the normal values of the relative weight of the liver.

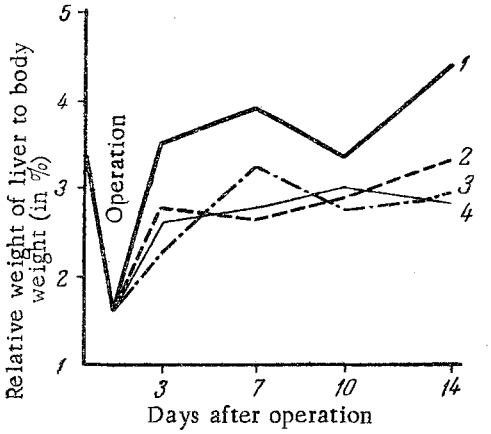


Fig. 1. Changes in the relative weight of the liver in the course of its reparative regeneration. 1) Injection of glycogen; 2) injection of physiological saline; 3) injection of glucose; 4) control.

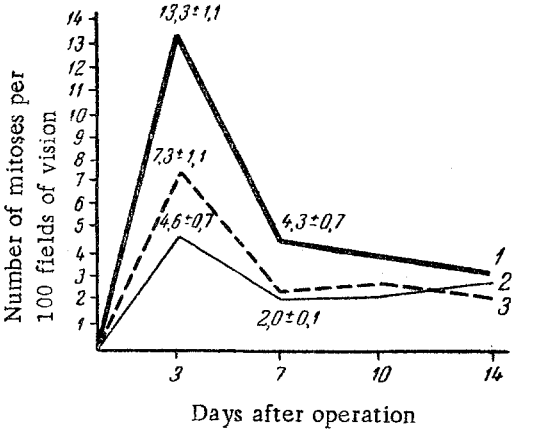


Fig. 2. Changes in the mitotic activity of the liver cells in the course of reparative regeneration of the liver. Along the axis of ordinates—number of mitoses in 100 fields of vision. 1) Injection of glycogen; 2) injection of glucose; 3) control.

Analysis of the gravimetric data thus showed that the relative weight of the liver in the rats receiving subcutaneous injections of glycogen rose much more sharply than the relative weight of the liver in the rats of all other groups. This result demonstrates that the subcutaneous injection of glycogen during the first week after operation has a well marked stimulating action on regeneration of the liver. The curves of the change in the relative weight of the liver in the control animals and in the rats of the groups receiving injections of physiological saline and glu-

cose almost coincided with each other, and the differences that did exist between them were not statistically significant. This indicates that the procedure of subcutaneous injection of physiological saline and glucose, in the doses which we used, itself has no essential effect on the course of regeneration of the liver.

We were unable to discover any differences in the histological structure of the regenerating liver in the rats of the different groups.

As in the previous investigation [2], three days after the operation we observed a marked inflammatory reaction at the periphery of the foci of necrosis of the liver parenchyma. The cytoplasm of the surviving liver cells, especially at the periphery of the lobules, contained fine granules and was infiltered with large droplets of lipid. Proliferative changes were observed in the form of the mitotic division of the liver cells. Seven days after the operation the beginning of the formation of zones of marginal necrosis was observed. The fatty infiltration of the surviving liver tissue was less well defined, and consisted of small droplets. Fourteen days after operation the normal lipid content of the liver was restored and the connective-tissue organization of the necrotic areas was complete. The liver showed little difference from normal, as regards both structure and lipid content.

Counts of the number of mitoses in the liver cells of rats sacrificed 3 days after operation revealed a statistically significant difference between that number in the rats receiving glycogen and in the rats of the other groups. The number of mitoses per 100 fields of vision in the liver of the rats receiving glycogen was 13.3 ± 1.1, whereas in the rats of the other groups the mitotic activity at this period did not exceed 7.3 ± 1.1, i.e., it was about half (Fig. 2).

These figures show that the more intensive increase in the relative weight of the liver in the process of preparative regeneration of the liver in the rats receiving glycogen was accompanied by greater mitotic activity of the liver cells.

In order to study the glycogen concentration in the liver of rats in the course of regeneration of that organ after subcutaneous injection of glycogen, we carried out a series of experiments on 106 adult male albino rats.

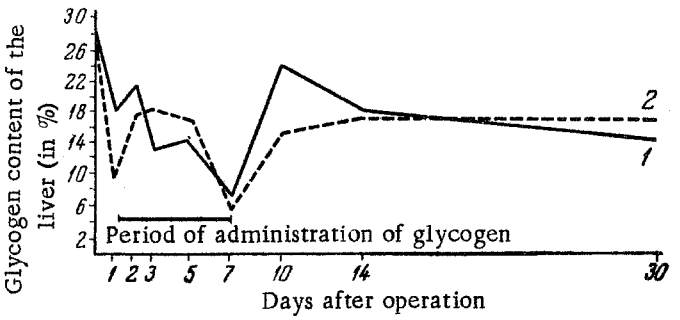


Fig. 3. Changes in the glycogen content of the liver in the course of its reparative regeneration. 1) Injection of glycogen; 2) control.

Ten of these rats were sacrificed to determine the glycogen content of the liver of normal animals, and the operation of partial resection of the liver was performed on the other 96 rats. After the operation, 48 rats received daily subcutaneous injections of glycogen (each of 4 ml of 45% glycogen solution in physiological saline) for one week, while the other 48 rats received no additional treatment. The dose of glycogen was increased in order to facilitate detection of the effects of the subcutaneous injection of glycogen on its concentration in the liver during reparative regeneration.

The rats were sacrificed 1, 2, 3, 5, 7, 10, 14, and 30 days after the operation, 6 rats from each group on each of these days. The glycogen content of the liver was determined by a gravimetric method after extraction from the liver with trichloroacetic acid and precipitation with alcohol. The results of these experiments are shown in Fig. 3.

It can be seen from Fig. 3 that no significant difference could be found in the glycogen content of the liver in the animals receiving glycogen and in the liver of the control animals. Until the 7th day after the operation a decrease in the glycogen content of the liver was observed both in the animals receiving glycogen and in the controls. The values of the minimal glycogen content in the animals of both groups, found on the 7th day after opera-

tion, were very close. The only feature of note was a greater increase in the glycogen content of the liver of the animals receiving glycogen than in that of the control animals on the 10th day after operation, when the glycogen content of the liver began to increase.

We thus were unable to detect any appreciable changes in the glycogen content of the regenerating liver of rats after the subcutaneous injection of glycogen.

Since we could find no information regarding the subcutaneous injection of glycogen in the accessible literature, we decided to study its pharmacological action. In acute experiments conducted on rabbits in conjunction with A. V. Smirnova, the subcutaneous and intravenous injection of glycogen, dissolved in physiological saline, in doses of 0.4-0.8 g/kg body weight, was tested with kymographic recording of the arterial pressure, pulse, and respiration. The results of these experiments showed that glycogen caused a slight and transient fall of the arterial pressure and slowing of the respiration, with a decrease in the amplitude of the respiratory movements. After the intravenous injection of glycogen a more rapid restoration of the initial level of the arterial pressure, pulse rate, and amplitude of the respiratory movements was observed.

We then injected glycogen in physiological saline subcutaneously in a dose of 0.4 g/kg body weight into healthy monkeys. No perceptible change in the arterial pressure or the pulse and respiration rates was observed.

In these experiments we were thus unable to detect any harmful action on animals resulting from the subcutaneous injection of glycogen.

Our results show that the subcutaneous injection of glycogen at a time when its content in the liver has fallen sharply after partial resection of the organ has a stimulating effect on the course of regeneration. The more intensive course of regeneration in the liver of the rats after the subcutaneous injection of glycogen is evidently associated with the more intensive multiplication of its cells, as shown by the findings in respect of the mitotic activity of the liver cells. Meanwhile the mechanism of stimulation of cell proliferation in the liver by the subcutaneous injection of glycogen remains unexplained.


## SUMMARY

Regeneration of the liver (following resection of $\frac{2}{3}$ of this organ) was stimulated by subcutaneous administration of glycogen at the period of marked drop of its content in the liver (during the first week after the operation). A more intensive regeneration in the rat liver following subcutaneous injection of glycogen is connected with increased cellular multiplication, as shown by the data on mitotic activity of hepatic cells. There were no significant changes in the glycogen content of the regenerating liver of rats following subcutaneous administration of glycogen. No deleterious effect of glycogen injections on the organism was detected.


## LITERATURE CITED


1. B. P. Solopaev, In: Problems of Regneration and Cell Division [in Russian], Moscow (1959), p. 68.
2. B. P. Solopaev, In: Problems in Reparative and Physiological Regeneration [in Russian], Moscow (1960), p. 64.
3. K. Aterman, Arch. Path. (1952), Vol. 53, p. 197.
4. R. D. Harkness, J. Physiol. (London) (1952), Vol. 117, p. 267.
5. H. O. Yokoyama, K. K. Tsuboi, M. E. Wilson, et al., Lab. Invest. (1953), Vol. 2, p. 91.


---



---